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Sugarcoating ER Stress

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The hexosamine biosynthetic pathway (HBP) generates metabolites for protein N- and O-glycosylation. Wang et al. and Denzel et al. report a hitherto unknown link between the HBP and stress in the endoplasmic reticulum. These studies establish the HBP as a critical component of the cellular machinery of protein homeostasis.

The hexosamine biosynthetic pathway (HBP) converts glucose to UDP-N-acetylglucosamine (UDP-GlcNAc) for N-glycosylation of proteins in the endoplasmic reticulum (ER) and dynamic O-glycosylation of cytosolic and nuclear proteins. The latter modification occurs on the hydroxyl groups of serine and threonine residues and serves regulatory functions, whereas the former occurs on specific asparagine residues and is important in the folding, stability and transport of proteins through the secretory pathway. In this issue of *Cell*, Wang et al. (2014) and Denzel et al. (2014) report a previously unknown link between the HBP and the unfolded protein response (UPR), a signaling network that is activated by protein folding stress in the ER to re-establish protein homeostasis (proteostasis) (Walter and Ron, 2011). Intriguingly, the HBP is directly activated by the UPR transcription factor XBP1s, which is induced upon ER stress by splicing of XBP1 mRNA. Upregulation of the HBP then stimulates cytoprotective mechanisms and longevity (Wang et al., 2014; Denzel et al., 2014).

O-glycosylation with GlcNAc is a modification that regulates cytosolic and nuclear protein function in a manner similar to phosphorylation. Increased levels of O-GlcNAc have been observed in cardiomyocytes after ischemia-reperfusion (I/R) and are associated with cardioprotection (Ngoh et al., 2011). After ischemia, initial damage is caused by reperfusion when sudden restoration of blood flow causes oxidative and nitrosative stress and Ca^{2+} overload. Stress-induced O-GlcNAc modifications promote cell survival by regulating processes, including Ca^{2+} homeostasis, defense against reactive oxygen species, and the heat shock response (Groves et al., 2013). Interestingly, I/R has been reported to induce the UPR (Llorente et al., 2013); however, a causal link between increased O-glycosylation and the UPR was so far unknown. To identify factors responsible for enhanced O-GlcNAc modification during I/R in murine models, Wang et al. (2014) investigated whether UPR activity is involved in regulating the HBP. Intriguingly, they found that XBP1s transcriptionally activates GFAT1, the rate-limiting enzyme of the HBP (Figure 1). UPR activation and increased O-GlcNAc modification occurred concurrently under various stress conditions besides I/R, suggesting a universal stress response mechanism. This notion was further supported by the finding that expression of XBP1s itself, in the absence of ER stress, led to HBP activation. Moreover, XBP1s was required and sufficient for cardioprotection in a GFAT1-dependent manner. Protection was also observed when cells were supplemented with GlcNAc, confirming HBP products as the critical factor. Thus, key HBP enzymes are directly induced by the UPR and stimulate cardioprotection during I/R (Figure 1).

The study by Denzel et al. (2014) sheds light on the downstream consequences of HBP induction during ER stress. To identify genes involved in regulating ER proteostasis, these authors performed a screen for mutants of *C. elegans* that were resistant to tunicamycin (Tm), an inhibitor of enzymes involved in N-linked glycosylation. They discovered a gain-of-function mutant of *gfat-1* (*gfat-1 gof*) that caused increased HBP activity, suppressed Tm toxicity and led to extended lif span. Addition of GlcNAc to wild-type worms or overexpression of GFAT-1 also led to Tm resistance and lifespan extension, demonstrating that the observed phenotype was in fact a consequence of increased HBP activity. Importantly, this phenotype was independent of known longevity factors of the insulin-like signaling pathway (DAF-2 and DAF-16), and no increase in HBP metabolites was observed in a set of long-lived mutants, including *daf-2*. Thus, the HBP emerges as a novel independent longevity pathway in *C. elegans*.

HBP activation was protective against disease-related aggregation-prone proteins, reducing the accumulation and aggregation of mutant neuroserpin protein SRP-2 in the ER and significantly improving motility of *C. elegans* expressing ER-targeted Aβ42 (Denzel et al., 2014). Notably, increased HBP activity not only improved ER proteostasis but also reduced the toxicity of disease-related aggregating proteins in the cytosol, indicating a mechanism of protection across different cellular compartments. Interestingly, the *gfat-1 gof* mutation, as well as exogenous addition of GlcNAc, led to upregulation of SEL-1—part of the ER ubiquitin-ligase complex required for the degradation of misfolded proteins by ER-associated degradation (ERAD)—and also increased proteasome activity and autophagy. Strikingly, overexpression of SEL-1 was sufficient to extend lifespan. Thus, by increasing ERAD capacity, as well as proteasomal protein degradation and autophagy, the HBP improves proteostasis in both the ER and cytosol (Figure 1).

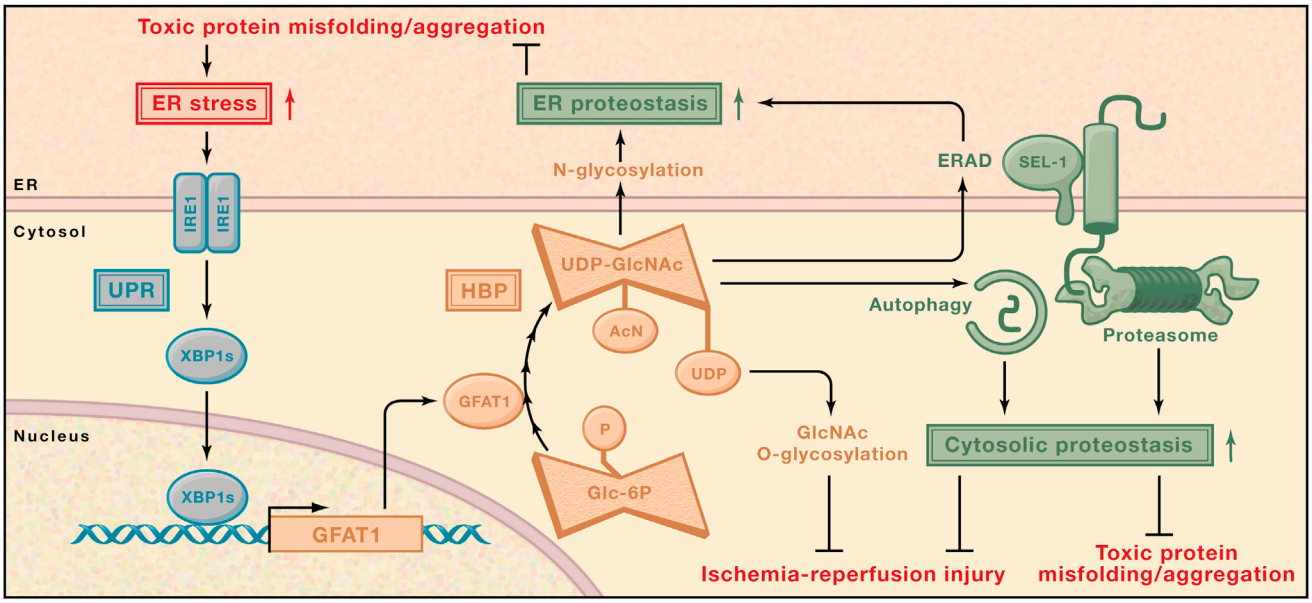

Figure 1. ER Stress-Induced HBP Leads to Proteostasis Recovery

Protein folding stress in the ER activates the ER transmembrane protein IRE1 as part of the UPR. IRE1 induces the transcription factor XBP1s by splicing XBP1 mRNA. XBP1s transcriptionally activates key enzymes of the HBP, including GFAT-1, leading to enhanced HBP activity and production of UDP-GlcNAc for the N-glycosylation and O-glycosylation of proteins. UDP-GlcNAc also stimulates proteasomal activity, induction of the ER-associated degradation (ERAD) component SEL-1, and autophagy. Increased HBP activity enhances proteostasis and mediates protection against toxic protein misfolding and aggregation in the ER and cytosol. UPR-mediated O-GlcNAc modification protects cells from damage caused by oxidative stress and Ca^{2+} overload after I/R.

The protective phenotypes observed by Denzel et al. (2014) and Wang et al. (2014) are a direct consequence of HBP activation by XBP1s. Interestingly, a recent report highlights the significant role of XBP1s for longevity in *C. elegans* (Taylor and Dillin, 2013). The new findings on HBP-mediated longevity now raise the question whether XBP1s-induced lifespan extension is generally mediated by the HBP. Denzel et al. (2014) report that induction of ER stress by Tm treatment of *C. elegans* also leads to GFAT1 expression, and Wang et al. (2014) observed induction of the murine GFAT1 after treatment with the ER stress inducer thapsigargin. Taken together, these findings strongly suggest that activation of the HBP is a critical component of the protective mechanisms that are orchestrated by XBP1s and lead to lifespan extension. Interestingly, GlcNAc has previously been reported to protect cells from the toxicity of glucose depletion by reducing proapoptotic UPR signaling (Palorini et al., 2013). Denzel et al. (2014) noted that, even though the *gfat-1 gof* phenotype was dependent on UPR function, *gfat-1 gof* did not activate the UPR. These findings are in line with HBP-mediated alleviation of ER stress and point toward a negative feedback loop.

The underlying molecular mechanisms by which an increase in HBP metabolites leads to improved proteostasis and cytoprotection are not yet fully understood. Consistent with the hypothesis that an increase in O-GlcNAc is the major protective factor after I/R, knockdown of the key enzyme driving this modification (OGT) in rat myocytes significantly reduced XBP1s-mediated protection (Wang et al., 2014). However, the specific O-GlcNAc-dependent proteins responsible for this effect remain to be identified. In contrast, Tm resistance and longevity in *gfat-1 gof C. elegans* were independent of the key enzymes that catalyze O-GlcNAc modification, pointing toward increased N-glycosylation as the main protective factor (Denzel et al., 2014). Thus, it is conceivable that both N- and O-glycosylation contribute to protection from cytotoxic insults with varying importance depending on the type of stress. In addition, some evidence suggests that the HBP-related enzyme GalE is transcriptionally activated by XBP1s (Wang et al., 2014). Notably, GalE drives UDP-GlcNAc conversion to UDP-GalNAc, the first monosaccharide added during O-glycosylation in the Golgi. Does ER stress-induced HBP activation also enhance O-glycosylation in the Golgi, and does this modification contribute to the protective effects of the HBP? ER stress-induced HBP activity may lead to a general improvement of cellular protein folding capacity via N-glycosylation but, in addition, may regulate specific cellular processes. Indeed, it is somewhat surprising that HBP activity causes induction of protein degradation pathways when considering that enhanced glycosylation should improve protein folding efficiency. Interestingly, Denzel et al. (2014) did not observe global changes in N-glycosylation upon HBP activation and suggest that proteins of the ERAD and autophagy machinery may be directly regulated by glycosylation. Thus, activating the clearance of misfolded proteins, rather than improving folding efficiency, may be the primary protective mechanism of the HBP upon ER stress.

The UPR and the HBP play important roles in neurodegenerative and metabolic diseases. The new studies highlight a

physiologically significant mechanistic link between these pathways and could open avenues to new therapeutic approaches. In particular, the protective effects of supplementation with HBP metabolites may represent a promising therapeutic strategy.

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Inflammasome: Putting the Pieces Together

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Microbial and danger signals result in inflammasome activation and release of inflammatory cytokines through mechanisms that remain elusive. Cai et al. and Lu et al. show that triggering of inflammasome sensors induces prion-like polymerization of the adaptor ASC into filaments. These structures function as platforms for inflammatory cytokine production and represent a unified mechanism for inflammasome assembly.

Inflammasomes are key signaling machines of the innate immune system that drive the production of the highly inflammatory cytokine interleukin-1β (IL-1β) via caspase-1 activation in response to microbial and nonmicrobial danger signals (Schroder and Tschopp, 2010). They are composed of a danger sensor, an adaptor protein—often ASC—and caspase-1. Prototypical inflammasome sensors, such as NLRP3 and AIM2, contain a pyrin domain (PYD) that can interact with the PYD of ASC (ASC^{PYD}), and ASC additionally possesses a caspase recruitment domain (CARD) for procaspase-1 engagement. Although the primary composition of the inflammasomes has been resolved and much of their physiological and pathological functions have been revealed through genetic studies, the biochemical and biophysical mechanisms of inflammasome activation remained mysterious. In this issue of *Cell*, two elegant reports demonstrate that AIM2 or NLPR3 triggering induces a prion-like polymerization of ASC into filaments that provide platforms for activating inflammatory cytokine production (Cai et al., 2014; Lu et al., 2014).

Prions were originally identified as the causative agents of spongiform encephalopathies in humans, but additional studies found prions also in lower organisms such as yeast (Prusiner, 1998). These proteins are functionally defined by their ability to induce an energetically favored self-polymerization process in which an initial nucleation step converts the native protein into the polymerized form. The Chen laboratory previously identified the immune adaptor MAVS as the first beneficial prion-like protein in mammals (Hou et al., 2011). After innate virus sensing, the RNA receptor RIG-I nucleates the N-terminal CARD of MAVS, which then undergoes prion conversion resulting in MAVS polymerization, NF-κB, and IRF3 activation. Now, the same group uses a yeast system to screen for prion-like features in additional immune signaling proteins that contain death domain (DD) folds, such as a PYD or CARD (Cai et al., 2014). By replacing the prion domain (NM) of the yeast Sup35 prion with candidate domains, the authors observe that the ASC^{PYD} exhibits remarkable prion-forming abilities. Interestingly, $NLRP3^{PYD}$ and AIM2 induce ASC^{PYD} prion conversion, and both AIM2